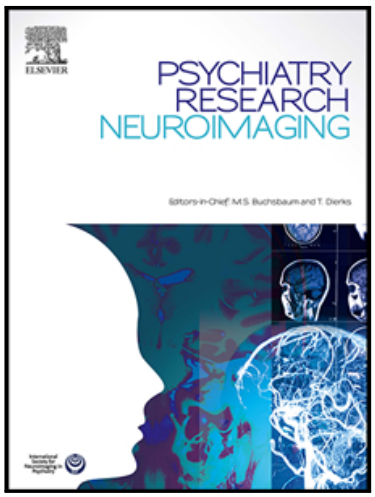

Increased [^{3}H]quisqualic acid binding density in the dorsal striatum and anterior insula of alcoholics: a post-mortem whole-hemisphere autoradiography study

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Highlights

- mGluR1/5 binding is increased in the putamen of Cloninger type 1 and 2 alcoholics
- mGluR1/5 binding is increased in the caudate head of both alcoholic subtypes
- mGluR1/5 binding is increased also in the anterior insula of both alcoholic subtypes
- In the hippocampus, mGluR1/5 binding is increased in Cloninger type 2 alcoholics

Increased [^{3}H]quisqualic acid binding density in the dorsal striatum and anterior insula of alcoholics: a post-mortem whole-hemisphere autoradiography study

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Abstract

The function of group I metabotropic glutamate receptors mGluR1 and mGluR5 is involved in the hyperglutamatergic state caused by chronic alcohol. Preclinical studies suggest that group I mGluR modulation could serve as a novel treatment of alcoholism. Considering the wide role of glutamatergic neurochemistry in addiction, group I mGluR binding was studied in brain areas involved in decision-making, learning and memory.

Post-mortem whole hemisphere autoradiography was used to study the binding density of [^{3}H]quisqualic acid, a potent group I mGluR agonist, in 9 Cloninger type 1 alcoholics, 8 Cloninger type 2 alcoholics and 10 controls. Binding was studied in the dorsal striatum, hippocampus and cortex.

Alcoholics displayed a trend towards increased [^{3}H]quisqualic acid binding in all brain areas. The most robust findings were in the putamen ($p = 0.006$) and anterior insula ($p = 0.005$), where both alcoholic subtypes displayed increased binding compared to the controls.

These findings suggest altered group I mGluR function in alcoholic subjects in the dorsal striatum, which is involved in habitual learning, and in the anterior insula, which has a pivotal role in the perception of bodily sensations. Increased [^{3}H]quisqualic acid binding might suggest a beneficial impact of mGluR1/5 modulators in the treatment of alcoholism.

Keywords: alcohol; autoradiography; dorsal striatum; insula; mGluR1; mGluR5

1. Introduction

Chronic alcohol use causes a hyperglutamatergic state, during which the amount of extracellular glutamate increases, and the function of the receptors and transporters involved in glutamate trafficking is altered (Holmes et al., 2013). Addictive drugs, including alcohol, affect glutamatergic synaptic plasticity in several brain regions. Altered glutamatergic state contributes to different characteristics of addiction; e.g., craving, devaluation of natural reinforcers, withdrawal and relapse (Kalivas and Volkow, 2005; Kauer and Malenka 2007). The glutamatergic system has an acknowledged role in the learning process (Anwyl, 2009; Galván et al., 2008; Yin and Knowlton 2006) and it has a broad regulatory role in cognitive functions (Terbeck et al., 2015).

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that modulate neurotransmission at a slower pace than ionotropic glutamatergic receptors. Group I mGluRs mGluR1 and mGluR5 mainly appear on postsynaptic structures (Hovelsø et al., 2012). Rodent studies identify intense group I mGluR distribution in the limbic areas that are essential in addiction; e.g., the hippocampus and dorsal striatum, and show substantial distribution in the cerebral cortex (Fotuhi et al., 1993; Martin et al., 1992; Romano et al., 1995; Shigemoto et al., 1992).

The impact of alcohol on group I mGluR function has been demonstrated in several animal studies. Chronic alcohol increases the levels of mGluR1 and mGluR5 proteins in several brain areas that are essential for both reward and craving, and also for learning and memory; e.g., the nucleus accumbens and dentate gyrus (Cozzoli et al., 2012; Galindo et al., 2004). Recent human PET studies have demonstrated alterations in mGluR5 availability in cortical and limbic areas of alcoholic subjects (Akkus et al., 2018; Leurquin-Sterk et al., 2018).

Group I mGluR modulators are an important research topic for the treatment of addiction, but also for the treatment of several other psychiatric disorders. In rodent studies, mGluR5 antagonists have attenuated withdrawal symptoms, reduced alcohol-intake and reduced reinstatement of alcohol-use. Preclinical and rodent studies suggest also anxiolytic and antidepressant effects for group I mGluR antagonists (reviewed by Holmes et al. 2013 and Krystal et al., 2010). Regretfully, studies with group I mGluR modulators have demonstrated also undesirable side-effects like devaluation of natural rewards and cognitive impairment (Holmes et al., 2013). In the light of the vast effect of group I mGluRs on various brain functions, the recent results of Phase I clinical trial testing of an mGluR5 negative allosteric modulator are promising, as the compound was well-tolerated (Haass-Koffler et al., 2017).

Several studies have demonstrated alcohol's ability to modulate synaptic plasticity (Kauer and Malenka, 2007), which is thought to cause the change from casual to compulsive alcohol use (Sjoerds et al., 2013). The diagnostic characteristics of addiction, like compulsive drug seeking and drug taking are thought to result from a process where repeated drug use leads to the formation of a habit. The function of the dorsal striatum has been suggested to play an essential role in this harmful learning process (Everitt and Robbins, 2005). mGluR1 and mGluR5 are strongly involved in LTP and LTD (long-term potentiation and long-term depression, respectively) in the hippocampus, striatum and neocortex (Galván, 2008; Gubellini et al., 2003; Lapointe et al., 2004; Perez et al., 2001; Sarihi et al., 2008). LTP and LTD are basic receptor level mechanisms underlying brain plasticity, which in turn is considered to be a major mechanism for learning and memory (Cooke and Bliss, 2006). Possible alterations in group I mGluR function in the striatal and hippocampal areas might be linked to pathological functional changes in these brain structures during addiction.

Previously, our research group has reported type-specific changes in the binding density of both ionotropic and metabotropic glutamate receptors of Cloninger type 1 and type 2 alcoholics. We have reported increased AMPA receptor and mGluR2/3 binding density in the anterior cingulate cortex and decreased NMDA receptor subunit NR2B binding in the nucleus accumbens of Cloninger type 2 alcoholics (Kärkkäinen et al., 2013; Kupila et al., 2015, Laukkanen et al., 2015) and increased group I mGluR binding density in the CA2 area of hippocampus of Cloninger type 1 alcoholics (Kupila et al., 2013). In Cloninger's model, alcoholics are divided into two subgroups. About 80% of alcoholics are Cloninger type 1 alcoholics, whose onset age of alcoholism is greater than 25 years. Typically, these people are low in novelty seeking, prone to anxiety, social and cautious characters. In contrast, Cloninger type 2 alcoholics, composing about 20% of alcoholics, are impulsive, high in novelty seeking and often comorbid with an antisocial personality disorder (Cloninger et al., 1988). The onset age of Cloninger type 2 alcoholism is usually less than 25 years. Group I mGluR function is involved in the regulation of stress and anxiety, which are among the main characteristics that separate Cloninger alcoholic subgroups. Glutamatergic neurotransmission in limbic areas is essential in the formation of anxiety (Riaza Bermudo-Soriano et al., 2012), and results of animal research highlight the role of mGluR5-driven signaling in both acute and chronic stress (Wagner et al., 2013; Wagner et al., 2015). In humans, mGluR5 availability in paralimbic and cortical areas associates with novelty seeking (Leurquin-Sterk et al., 2016).

Recent in-vivo neuroimaging human studies have investigated mGluR5 availability in patients with alcohol use disorder (AUD) (Akkus et al., 2018; Leurquin-Sterk et al., 2018). Previously, we have reported increased mGluR1/5 binding density in the hippocampus CA2

area of Cloninger type 1 alcoholics in a whole-hemisphere post-mortem autoradiography (Kupila et al., 2013). This preliminary study covered only the level of the ventral striatum, where no other differences in mGluR1/5 binding density were detected. In order to get a more complete picture of the possible changes in group I mGluR binding density, we now evaluated mGluR1/5 binding density in Cloninger type 1 and 2 alcoholics in brain areas that are relevant for habituation and learning, such as the dorsal striatum (Kalivas and Volkow, 2005), but also cortical areas and the hippocampus.

2. Materials and methods

2.1 Diagnostics and sample preparation

The methodology of this study has been described earlier (Kupila et al., 2013; Tupala et al., 2003). In brief, post-mortem brain left hemispheres (17 alcoholics and 10 controls) were obtained during clinical autopsy from the Department of Forensic Medicine, University of Oulu, Finland, and two of the non-alcoholic control brains were obtained from the Department of Forensic Medicine, University of Eastern Finland, Kuopio, Finland. The recovery procedure was essentially the same in both locations. This part of the study was approved by the Ethics Committees of the University of Oulu, Finland and the National Institute of Medico-legal Affairs in Finland. A post-mortem analysis for drugs, which included alcohol, and the normal necropsy protocol were performed. None of the hemispheres exhibited damage or gross neuro-anatomical abnormalities. Medical records on the cause of death, previous diseases, and medical treatments of the subjects were also collected.

Diagnoses were made by two physicians, independently from each other. Medical records' data were available for all 27 subjects. Mental disorders were coded according to DSM-IV criteria (APA, 1994), and alcoholic subjects were further sub-classified as type 1 or 2, according to criteria established by Cloninger (1988). The kappa coefficient of diagnostic agreement between the diagnoses was 0.9; i.e., one type 2 alcoholic was diagnosed as a type 1 alcoholic by the other physician. Otherwise, diagnoses were unanimous. The most important criteria for defining these two groups of alcoholic subjects were early onset of alcohol abuse (before the age of 25) and documented severe antisocial behavior for the Cloninger type 2 alcoholic subjects. Subjects having psychotic disorders or any neurological diseases (such as epilepsy), or those taking medication that could affect the CNS (such as neuroleptics or antidepressants), were excluded. A history of tobacco smoking, based only on medical records, was considered unreliable and was not included in the final criteria.

All 27 subjects were Finns. The study groups consisted of nine type 1 alcoholic subjects (seven men and two women; age: mean= 52.7 years, standard deviation (SD)= 12.4; post-mortem interval (PMI): mean= 11.9 hours, SD= 4.5); eight type 2 alcoholic subjects (all men; age: mean= 34.6 years, SD= 12.2; PMI: mean= 14.1 hours, SD= 3.4); and 10 non-alcoholic control subjects (eight men and two women; age: mean= 53.5 years, SD= 10.7; PMI: mean= 14.8 hours, SD= 9.2). One of the controls was excluded from the analyses as a statistical outlier. None of the controls had a psychiatric diagnosis. There was no statistically significant difference in the time between death and autopsy between the three groups (P= 0.62 – 0.98, Scheffe's test for multiple comparisons, two-tailed). Six of the eight type 2 alcoholic subjects had a criminal record or a history of violent offences (physical or sexual). Alcoholism in both type 1 and type 2 groups was severe, as judged by frequent admissions to emergency stations

and doctors' appointments due to alcohol-related problems. Eight of the nine type 1 alcoholics had alcohol in their blood at the time of death, and one alcoholic subject had an incarcerated abstinence period of 10 hours. Two type 1 alcoholic subjects had traces of diazepam in their blood samples. Six of the eight type 2 alcoholic subjects had alcohol in their blood at the time of death, three had traces of benzodiazepines, and one tested positive for cannabinoids, however this may have been a false positive due to the use of ibuprofen, with the diagnostic test that was used. One control subject had a small amount of alcohol in his blood at the time of death (0.04%). All subjects died of sudden causes. For details of the study subjects, see Supplementary Information, Table S1.

Cryosectioning and autoradiography were performed at the Department of Pharmacology and Toxicology, University of Eastern Finland, Kuopio, Finland (for detailed methods, see Tupala et al., 2003). Individual variations in brain size were considered when selecting representative sections. Each cryosection was coded for a subsequent blind analysis of the data.

2.2 Group I mGluR binding assay and data analysis

We used tritium labeled quisqualic acid ([^{3}H]quisqualic acid) in human post-mortem whole hemisphere autoradiography. The method of this study was identical to our earlier mGluR1/5 binding density study (Kupila et al., 2013), and a modification of that reported by Mutel and his co-workers (2000), and of our previous experiments with other radioligands for the same study subjects (for example, see Laukkanen et al., 2013). Two parallel incubations were performed in containers of 2.5 liters, both consisting of one canto-meatal brain slice of each 27 study subjects. All cryosections were pre-incubated for 2 x 10 minutes in the 50mM Tris-

HCl buffer, pH 7.4, containing 2mM $MgCl_2$, 2mM $CaCl_2$ (at room temperature). To reach equilibrium, one set of 27 sections was incubated for 60 minutes at room temperature in a solution containing 3nM of [^{3}H]quisqualic acid (Code TRK1070, Batch 24, Specific activity = 28.0 Ci/mmol, GE Healthcare UK Ltd. Amersham, UK). Non-specific binding was determined by incubating a set of 27 adjacent sections with 10µM glutamate as a displacer. Both incubations contained 30µM kainate to inhibit [^{3}H]quisqualic acid binding to ionotropic glutamate receptors. In the method paper of Mutel and colleagues (2000), the Kd values of [^{3}H]quisqualic acid were reported to be 27 and 81 nM for mGluR1 and mGluR5, respectively. Cryosections were washed in a cold buffer for 3 x 1 min, followed by a brief dip into ice-cold distilled water. Thereafter, sections were dried under a gentle stream of warm air for 10 minutes and then left for 5 days at room temperature before exposure to phosphor imaging plates (BAS IP-TR 2040, Fuji Photo Film, Co., LTD, Japan) for 14 days, and subsequently scanned (Storm 860 PhosphorImager scanner, Amersham, Little Chalfont, UK). The autoradiograms were analyzed using phosphor imager analysis (Image J, National Institutes of Health, USA), and the resulting luminescent values of the binding data were converted to pmol/mg by the use of ^{3}H-calibrating scales (cat. no. RPA 507, Amersham, Little Chalfont, UK). The non-specific binding was subtracted from the total binding to yield specific binding. All analyses were made blind to the clinical classification of the sample.

In all, twelve brain areas from the canto-meatally oriented level of the dorsal striatum were selected for the analysis; i.e., the anterior cingulate cortex, the anterior prefrontal cortex, the medial prefrontal cortex, the lateral prefrontal cortex, the posterior cingulate cortex, the hippocampus, the dentate gyrus, the anterior insula and the posterior insula, the head of the caudate, the putamen and the globus pallidus.

2.3 Statistical analyses

Results are expressed as the mean with SD. Due to the small sample size, the data was unsuitable for straightforward statistical analyses. The statistical significance between groups was evaluated by a bootstrap type analysis of co-variance (ANCOVA, 5000 replications) with appropriate contrast (Efron and Tibshirani, 1998), using age as a covariate. A bootstrap-based multiplicity adjustment was applied to correct the levels of significance for multiple testing. Possible correlations between [3H]quisqualic acid binding and age, PMI and blood alcohol content (BAC) were determined by a two-tailed Pearson's correlation coefficient. P-values of less than 0.05 were considered to be statistically significant for all tests. STATA (release 11.2, College Station, TX) was used for statistical analyses.

3. Results

The [^{3}H]quisqualic acid binding to brain areas can be observed in Figure 1, and the results of selective [^{3}H]quisqualic acid binding are presented in Table 1. Both alcoholic subtypes displayed increased binding in all of the 12 measured brain areas when compared to controls. There was a statistically significant difference in [^{3}H]quisqualic acid binding density between three study groups in the dorsal striatum, in the putamen ($p = 0.006$) and in the caudate head ($p = 0.041$), where both Cloninger type 1 and type 2 alcoholics displayed significantly increased binding density when compared to controls. There also was a statistically significant difference in [^{3}H]quisqualic acid binding between the groups in the anterior insula ($p = 0.005$), where both alcoholic subgroups displayed significantly increased [^{3}H]quisqualic acid binding. In the hippocampus, [^{3}H]quisqualic acid binding between the study groups differed significantly ($p = 0.029$), and the increase in binding density of Cloninger type 2 alcoholics was significant. Age, PMI or BAC did not correlate with [^{3}H]quisqualic acid binding.

4. Discussion

The main finding of this study is a statistically significant increase in [^{3}H]quisqualic acid binding density, indicating increased mGluR1/5 binding, in the putamen, caudate head and anterior insula of Cloninger type 1 and Cloninger type 2 alcoholics. A statistically significant increase in mGluR1/5 binding was detected in the hippocampus of Cloninger type 2 alcoholics (See Figure 2). There was a global trend of increased binding density in both alcoholic subgroups (See Table 1). The main limitation of our study is the small number of study subjects. Therefore, these results should be considered as preliminary, and need to be confirmed by future studies.

4.1 A trend of increased mGluR1/5 binding density in alcoholics

Human and animal in-vivo imaging studies have reported alcohol-induced alterations in mGluR5 availability, but the results are partly conflicting. A recent human PET study reported increased mGluR5 availability in the temporal lobe, but not in the basal ganglia (Akkus et al., 2018), whereas another study (Leurquin-Sterk et al., 2018) found decreased mGluR5 availability in the limbic region of abstinent alcoholics. A rodent PET study suggested increased mGluR5 availability in cortical and hippocampal areas right after alcohol dosing (Nandi et al., 2016). Taken together, the results of imaging studies highlight the dynamic effect of alcohol on synaptic plasticity and receptor expression. In our post-mortem autoradiography study, we found a trend of increased mGluR1/5 binding density in Cloninger type 1 and type 2 alcoholics. Due to the methodological differences and patient selection, our results cannot be compared with the findings of in-vivo imaging studies. However, our finding suggests involvement of group I mGluRs in alcoholism.

4.2 Increased mGluR1/5 binding in the dorsal striatum of alcoholics

We found a significant increase in [^{3}H]quisqualic acid binding in the putamen and caudate head of both alcoholic subgroups when compared to the controls. The human caudate is connected to the associative cortices and the hippocampus, and thus involved in goal-directed learning, whereas the putamen connects to the sensorimotor cortices, as an area of habitual learning (Yin and Knowlton, 2006). A human fMRI study of Tricomi and co-workers (2009) found strengthening BOLD (blood oxygen level dependent) activation of the posterior putamen during habit formation, and animal and human studies report activation of the dorsal striatum in chronic substance abuse (Porrino et al., 2004; Sjoerds et al., 2013). mGluR5 and mGluR1 are vital parts of LTP and LTD of the striatum and corticostriatal connections enabling brain plasticity required in a learning process (Anwyl, 2009; Cooke and Bliss, 2006; Gubellini et al., 2003). The increased mGluR1/5 binding observed in our study may affect LTP and LTD processes of the dorsal striatum and be linked to maladaptive habit learning in alcoholics.

4.3 Increased mGluR1/5 binding in the anterior insula of alcoholics

We found a statistically significant difference in [^{3}H]quisqualic acid binding density between the groups in the anterior insula. The increase in binding was statistically significant in Cloninger type 1 and 2 alcoholics.

The insular cortex is broadly connected to several cortical and subcortical areas (Augustine, 1996; Wisner et al., 2013). The anterior insula is activated in effortful cognitive processing and cognitive perception of bodily states, and the intensity of urge in addiction correlates with activation of the insula (Craig, 2002; Naqvi and Bechara 2010; Paulus and Stewart, 2014). Human fMRI studies have demonstrated imbalanced connections of the insula with

several other brain areas in addiction, and in a PET study, mGluR5 availability of the insula correlated positively with the mGluR5 availability in the other cortical regions in AUD patients, but not in the controls (Akkus et al., 2018; Claus et al., 2013; McHugh et al., 2013; Wisner et al., 2013). The increased mGluR1/5 binding in the anterior insula of alcoholics observed in our study might affect the cognitive interpretation of bodily states, which has been linked to addiction (Naqvi and Bechara, 2010).

4.4 Increased mGluR1/5 binding in the hippocampus of Cloninger type 2 alcoholics

In the hippocampus, Cloninger type 2 alcoholics displayed a statistically significant increase in [^{3}H]quisqualic acid binding density when compared to controls. This finding needs confirmation of further studies, for we had to exclude 2 controls and 2 type 1 alcoholics from the analysis of hippocampus due to anatomical and technical problems. In addition, the heterogeneity among type 1 alcoholics was wide. Recent rodent studies suggest alcohol-induced alterations in hippocampal mGluR5 expression, which seem to progress during different states of intoxication and withdrawal (de Laat 2018 et al., Marszalek-Grabska et al. 2018). Our findings of altered mGluR1/5 binding in the hippocampus of Cloninger type 2 alcoholics is in line with these studies, suggesting altered group I mGluR function of this area in alcoholism.

4.5. A comprehensive interpretation of our mGluR1/5 study results

In our previous study about [^{3}H]quisqualic acid binding density on the level of ventral striatum, we found increased mGluR1/5 binding density in the CA2 area of hippocampus in Cloninger type 1 alcoholics, and no further differences between the groups were detected (Kupila et al., 2013). In that study, one area of the ventral striatum was defined as nucleus accumbens, beginning at the level where the caudate nucleus and putamen unite. The

remnants of the fused caudate and putamen were also quantitated, even though such division may not be anatomically justified, and these anatomical terms should be used only on the level of the dorsal striatum (Neto et al., 2008). In order to get a more complete picture of the possible alterations in mGluR1/5 binding in Cloninger type 1 and 2 alcoholics, we now analyzed [^{3}H]quisqualic acid binding in the level of dorsal striatum, and detected increased mGluR1/5 binding in both alcoholic subgroups in the putamen, head of caudate and anterior insula of both alcoholic subgroups. In addition, we observed increased mGluR1/5 binding in the hippocampus of Cloninger type 2 alcoholics. In the current study, we did not divide hippocampus to the subregions of CA1, CA2 and CA3. This approach was chosen, for the anatomical areas of these subregions are small and their borders partly difficult to define. In our previous study, binding densities of CA1 and CA2 were highly similar and correlated in all study groups, and in the controls, binding of all three subregions correlated positively. Even though the hippocampus was analyzed as a whole, we had to exclude 4 subjects from the analysis due to technical difficulties. Therefore, the findings of this area need to be interpreted with caution.

It has been suggested, that during different stages of addiction, different parts of the striatum are activated. There is a gradual shift in activity from the ventral to the dorsal striatum during the addictive process. At the same time, goal-oriented action, which is sensitive to the variable cues of the environment, is taken over by habitual automatic behavior. This, in turn, is characteristic to addiction and other binge-type repetitive behaviors seen in many psychiatric and neurologic conditions (Graybiel, 2008). Before their death, the alcoholism of our alcoholic study subjects was severe and, in most cases, it had endured for years. Together, the findings of our previous (Kupila et al., 2013) and this study may suggest altered group I mGluR function in the dorsal striatum, but not in the ventral striatum of alcoholics

when compared to controls. Our study method can't distinguish, whether this alteration in mGluR1/5 binding density preceded alcoholism, or is a consequence of the repetitive behavior, the prolonged severe alcohol use.

5. Conclusions

In conclusion, we used post-mortem human whole hemisphere autoradiography to study [^{3}H]quisqualic acid binding density in Cloninger type 1 and 2 alcoholics and controls. The most robust, statistically significant increase in binding density was detected in the dorsal striatum and anterior insula of both alcoholic subgroups. The increase in mGluR1/5 binding in the putamen and caudate head might link to the tendency of a habituated response among alcoholics, which maintains addictive behavior and predisposes to relapse. The increased mGluR1/5 binding in the anterior insula might affect the function of this area, like cognitive processing of bodily states and urges, which links to withdrawal and relapse propensity. These findings suggest that group I mGluRs may be a useful target for pharmacological therapy of alcoholism.

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TABLE 1.

Table 1. Selective and specific [^{3}H]quisqualic acid binding density to mGluR1/5 in controls, Cloninger type 1 and Cloninger type 2 alcoholics in twelve brain areas on the canto-meatally oriented level of the dorsal striatum.

Brain area of interest,	**Controls** Mean (SD)	**Type 1** Mean (SD)	**Type 2** Mean (SD)	**p-value**
aPFC	5.23 (0.92)	6.48 (2.45)	6.48 (2.45)	0.22
mPFC	4.96 (1.09)	5.83 (2.17)	7.07 (3.20)	0.13
lPFC	6.68 (2.24)	10.88 (7.19)	9.69 (4.87)	0.10
ACC	6.25 (1.37)	7.06 (2.01)	8.07 (4.40)	0.29
aINS	**7.48 (1.98)**	**11.17 (4.61)***	**11.18 (5.01)***	**0.005**
pINS	7.92 (1.84)	11.18 (5.44)	11.88 (6.17)	0.053
Putamen	**3.00 (0.77)**	**4.38 (1.80)***	**4.23 (1.69)***	**0.006**
Head of Caudate	**4.23 (1.03)**	**5.67 (2.04)***	**5.49 (2.37)***	**0.041**
GP	0.75 (0.55)	1.32 (0.91)	0.86 (0.72)	0.089
Hippo	**8.55 (2.29)**	**13.83 (7.18)**	**12.74 (4.24)***	**0.029**
DG	7.60 (2.01)	8.25 (3.61)	9.21 (2.82)	0.17
PCC	4.51 (2.17)	7.53 (4.54)	8.33 (5.50)	0.057

TABLE LEGEND

Legends: Mean stated as the mean binding density (pmol/mg) of [^{3}H]quisqualic acid; SD, standard deviation; ACC, anterior cingulate cortex; aINS, anterior insula; aPFC, anterior prefrontal cortex; DG, dentate gyrus; GP, globus pallidus; Hippo, hippocampus; lPFC, lateral prefrontal cortex; mPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; pINS, posterior insula; *, statistically significant difference when compared to controls (bootstrap type analysis of co-variance, 5000 replications, using age as covariate, with a bootstrap-based multiplicity adjustment as a correction for multiple testing).

FIGURE LEGENDS

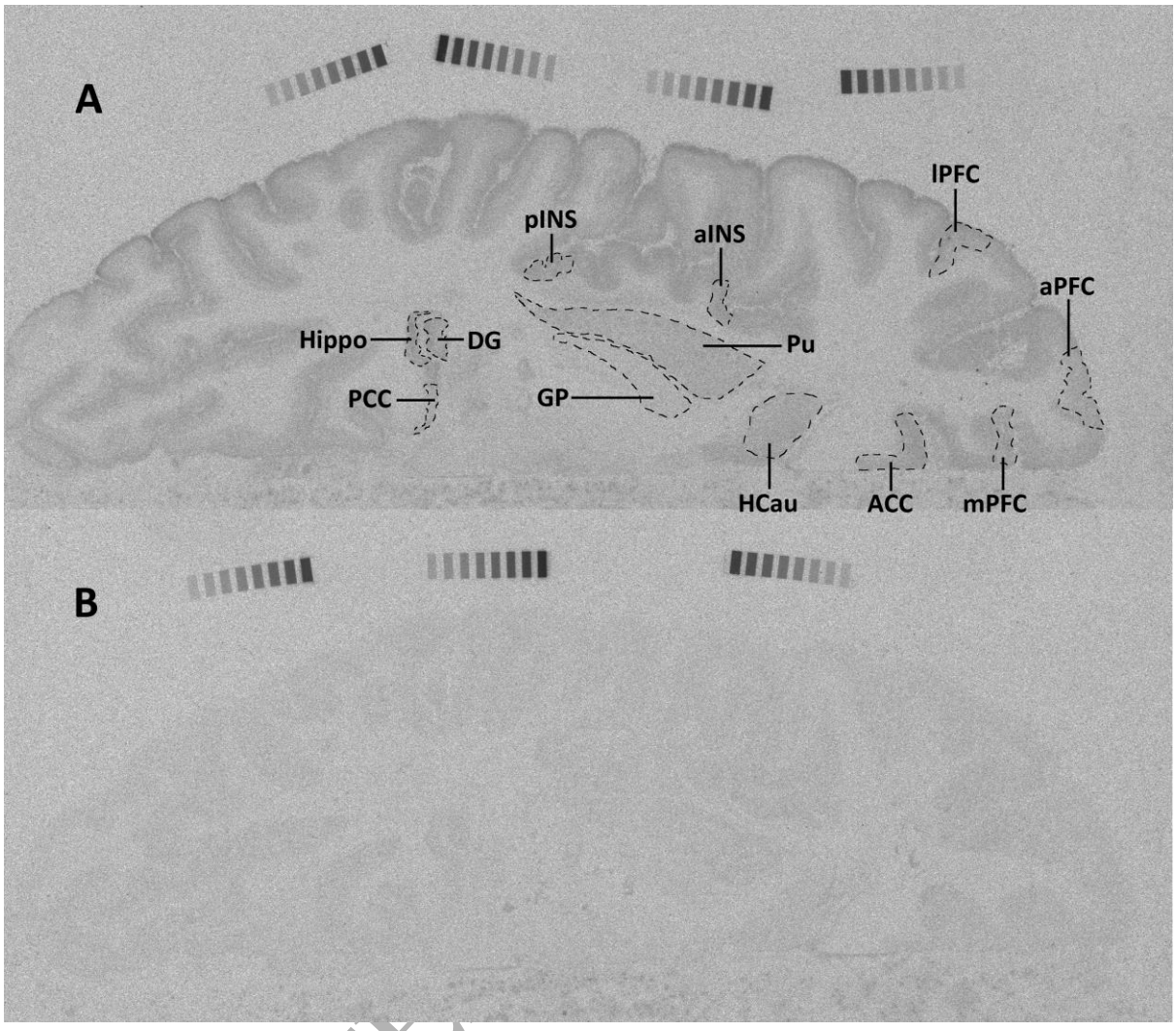

Figure 1. The whole brain hemisphere autoradiograms of the canto-meatally oriented brain slices on the level of the dorsal striatum, demonstrating A) the selective binding of [^{3}H]quisqualic acid without a displacer, and B) [^{3}H]quisqualic acid binding with gluatamate as a displacer. Legends: ACC, anterior cingulate cortex; aINS, anterior insula; aPFC, anterior prefrontal cortex; DG, dentate gyrus; GP, globus pallidus; HCau, caudate head; Hippo, hippocampus; lPFC, lateral prefrontal cortex; mPFC, medial prefrontal cortex; PCC posterior cingulate cortex; pINS, posterior insula; Pu, Putamen.

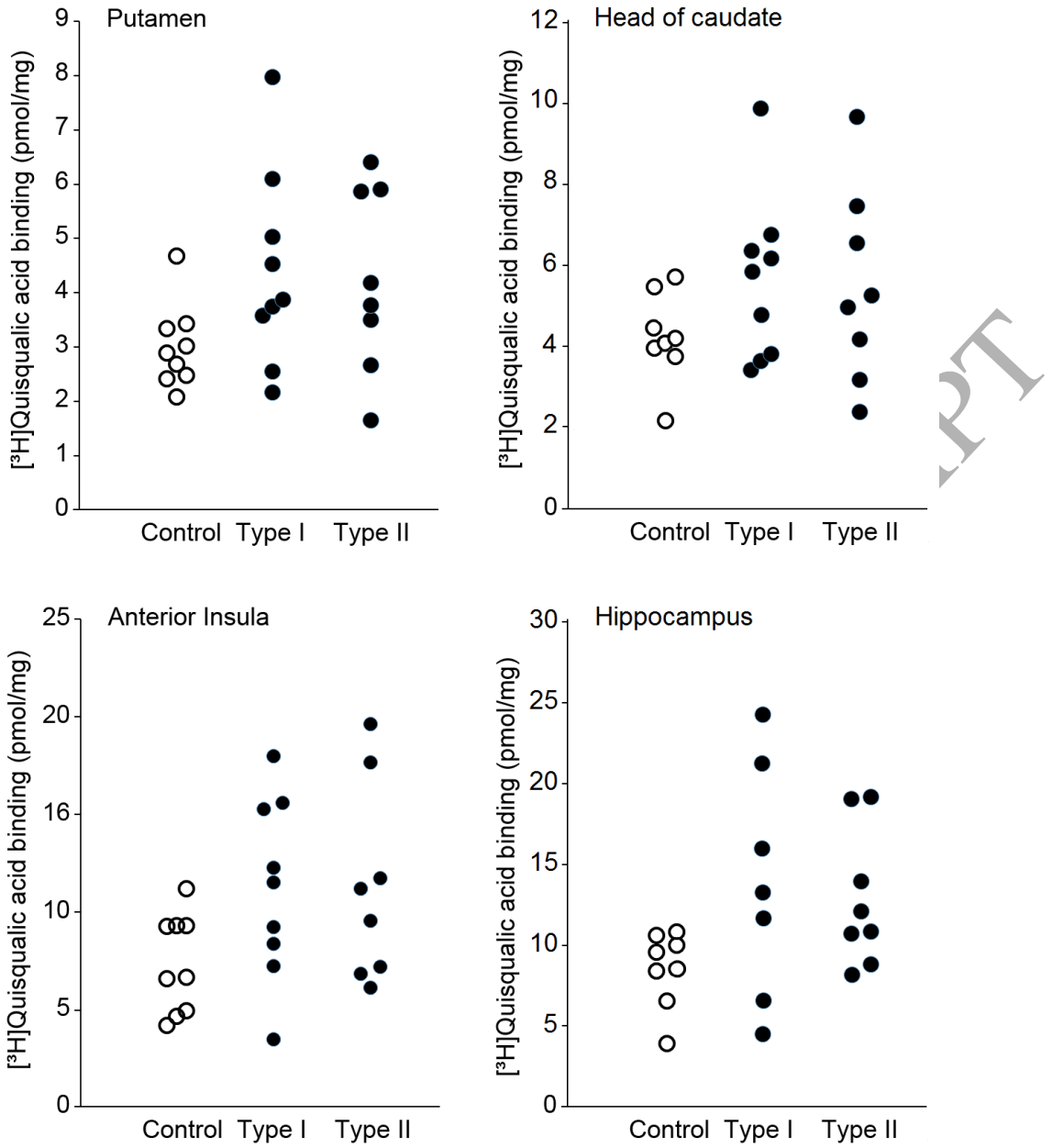

Figure 2. Selective and specific [³H]quisqualic acid binding to mGluR1/5 in controls, Cloninger type 1 and Cloninger type 2 alcoholics. The binding density was significantly different between three groups both in the putamen (p = 0.006), head of caudate (p = 0.041), anterior insula (p = 0.005) and hippocampus (p = 0.029) by using bootstrap-type analysis of covariance, 5000 replications, using age as covariate. Both alcoholic subgroups displayed a statistically significant binding increase when compared to the controls in the putamen, head of caudate and anterior insula, whereas in the hippocampus, the increase in binding in

Cloninger type 2 alcoholics was statistically significant. A greater heterogeneity among alcoholic subjects than controls can be observed.